

Chemical and Microbiological Safety of Burukutu: An alcoholic beverage vended in Owerri, Imo state, Nigeria

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ABSTRACT

Burukutu is a traditional alcoholic beverage voraciously consumed in Northern Nigeria and some other West African countries. It is made from fermenting sorghum (*Sorghum vulgare*) or millet (*Pennisetum maliaceum*) grains involving crude traditional methods of malting, mashing, steeping, fermentation and maturation. The microbial quality of *Burukutu* sampled at four locations in Owerri, Imo State, Nigeria was investigated using the spread plating technique. Total titratable acidity (TTA) and pH was determined under ambient temperature. Phytochemical constituents and antioxidant contents of the beverage were also analysed using standard methods. Total aerobic plate count, total coliform count, total fecal coliform count and total fungal count suggest cross contamination of the final products. Six Gram positive bacteria, namely, *Bacillus cereus*, *Staphylococcus aureus*, *Micrococcus luteus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Corynebacterium diphtheriae* and six Gram negative, *Escherichia coli*, *Enterobacter cloaca*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Serratia marcescens* were isolated from the *Burukutu* across the sample locations. *Mucor*, *Rhizopus* and *Saccharomyces cerevisiae* were the fungi isolated from the samples. The pathogenicity of some of these microorganisms had been widely reported. pH is acidic and may preserve the products and inhibit some pathogens. Titratable acidity is in acceptable limit recommended for alcoholic beverages. Phytochemicals present have been reported to exert strong antibacterial activity against several microbes associated with diseases. Antioxidant protects the body from oxygen scavenging bacteria. Most of the organisms isolated are indicators of poor hygiene practices, thereby compromising the safety of the product. Adherence to proper hygiene and good manufacturing practices and sensitization of workers and personnel to health risk associated with contamination of products is strongly recommended.

Keywords: Microbial diversity; Chemical composition; *Burukutu*; Total titratable acidity; Antibacterial activity; Oxygen scavenging bacteria.

1.0. Introduction

Developing countries like Nigeria depend mostly on indigenous technology for food preparations especially food of plant origin including alcoholic beverages made from cereal grains (Augustine *et al.*, 2016). Alcoholic beverages like *Burukutu*, *Pito*, *Sekete*, and others have been enjoyed for pleasure shortly after their production (Malomo & Popoola, 2020). The production and sale of these local brews also serve as a source of income (Augustine *et al.*, 2016).

Burukutu, a locally brewed nutritious alcoholic drink with a vinegar-like taste and a cloudy appearance, is primarily made from the grains of either *Sorghum bicolor* or *Sorghum vulgare* which are rich in energy and protein compounds (Lynn *et al.*, 2014; Atter *et al.*, 2014; Eze *et al.*, 2011; Kolawole *et al.*, 2018; Kamatchi *et al.*, 2010).

The major challenges associated with the production of *Burukutu* is the short shelf life and presence of diverse microbial communities that could be responsible for potential spoilage or contamination (Bala *et al.*, 2017; Egwurochi *et al.*, 2021; Eze *et al.*, 2011) resulting from poor sanitary processing (Lynn *et al.*, 2014). *Burukutu* is in demand as a substitute for beer by low income earners, thus necessitating an increase in production leading to a drop in the already low level of hygiene practices in the production which may create an avenue for the contamination and spread of microorganisms of public health significance (Eneji *et al.*, 2017; Anaukwu *et al.*, 2015).

This study evaluates the microbiological and chemical status of *Burukutu*, an alcoholic beverage sold in some communities in Imo State, Nigeria.

1.1. Study objectives

(i) Characterization and identification of microorganisms isolated from *Burukutu*; Phytochemical composition of *Burukutu*; Antioxidant content of *Burukutu*; Antibiotic susceptibility of commercial antibiotics against bacterial isolates.

(ii) Determination of pH, Temperature and Titratable acidity of *Burukutu*.

2.0. Materials and Methods

2.1. Sample collection and Preparation

Fresh samples were purchased in bottles and calabashes directly from the source of production and from vendors at Obinze, Nekede, Ihiagwa, Eziobodo and Ama-Hausa in Owerri, Imo State. Sample size was determined by the method of Kothari (2004).

2.2. Microbiological Analysis

2.2.1. Preparation and Inoculation of Samples and Determination of Bacterial Population

Fresh samples were prepared and serial diluted following standard methods. This was done using a digital colony counter and the total colony forming (CFU) expressed in milliliter of the sample (Cheesbrough, 2000).

2.2.2. Isolation, Characterization and Identification of Bacterial Isolates

Microbial isolates were characterized based on cultural (colonial), microscopic and biochemical methods with reference to standard manuals (Cheesbrough, 2000). The identities of the isolates were cross-matched with reference to standard manuals for the identification of bacteria (Buchanan & Gibbon, 2000).

2.2.3. Determination of pH

pH was determined more accurately by electrochemical methods using electrodes and a millivoltmeter (pH meter) previously calibrated (Somayeh *et al.*, 2018).

2.2.4. Determination of Titratable Acidity

This was determined by the methods described by neutralizing the acid in a known quantity of the sample using a standard base. The endpoint for titration is usually either a target pH or a colour change of a pH-sensitive dye, typically phenolphthalein (Nielson, 2017; Somayeh *et al.*, 2018; Atter *et al.*, 2014).

2.2.5. Determination of Antioxidant

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang *et al.* (2001). The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm.

2.2.6. Determination of Phytochemicals

Burukutu samples were screened for the presence of Alkaloids, Saponins, Tannins, Cardiac Glycoside, Anthraquinones, Steroid, terpenoid and flavonoids, according to standard methods (Edori *et al.*, 2019; Thilagavathi *et al.* 2015; Maria *et al.*, 2018).

3.0. Results

3.1. Enumeration and Morphological Identification

The total count of bacteria isolated from locally brewed alcoholic beverage (*Burukutu*) on different media is shown in Table 1. The total counts is in the range of 1.02×10^5 - 1.96×10^5 on nutrient agar; 2.0×10^4 - 1.27×10^5 on Eosin Methylene Blue Agar; 3.6×10^4 - 1.01×10^5 on Salmonella Shigella Agar and 1.16×10^6 - 1.55×10^6 on Mannitol Salt Agar. Samples from OBZA recorded more contaminants on all the media and NEKD the least.

Colonial and microscopic characteristics of the isolates is shown on Table 2. Five Gram positive, namely, *Bacillus cereus*, *Staphylococcus aureus*, *Micrococcus luteus*, *Enterococcus faecalis*, *Bacillus subtilis*, six Gram negative, *Escherichia coli*, *Enterobacter cloaca*, *Klebsiella pneumonia*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Serratia marcescens* and one Gram positive pleomorphic bacterium, *Corynebacterium diphtheriae* was isolated from the *Burukutu* across the sample locations. Identification was based on colonial, microscopic and few biochemical characteristics.

Table 1. Total count of bacterial isolates (CFU/ml)

Sample Location Code	NA	EMBA	SSA	MSA
OBZA	1.02×10^6 - 1.96×10^6	2.0×10^4 - 1.27×10^5	3.6×10^4 - 1.01×10^5	1.16×10^6 - 1.55×10^6
IHAG	2.8×10^5 - 9.8×10^5	1.6×10^4 - 7.5×10^4	1.0×10^4 - 3.7×10^4	1.7×10^4 - 5.5×10^5
NEKD	5.0×10^5 - 6.7×10^5	1.0×10^4 - 3.3×10^4	1.6×10^4 - 3.0×10^4	1.1×10^4 - 4.2×10^5
EZBO	4.0×10^5 - 6.1×10^5	2.9×10^4 - 3.9×10^4	1.2×10^4 - 6.1×10^4	3.9×10^4 - 7.1×10^5
OWER	2.8×10^5 - 3.7×10^5	1.9×10^4 - 5.9×10^4	2.8×10^4 - 4.4×10^4	3.3×10^4 - 3.5×10^5

NA, Nutrient Agar; EMBA, Eosin Methylene Blue Agar; SSA, Salmonella Shigella Agar; MSA, Mannitol Salt Agar.

Table 2. Colonial and Microscopic Characteristics of Bacterial Isolates

Colonial Morphology	Grams Morphology	Motility	Capsule Production	Spore Production	Probable Identity
Dull serrated flat cream colonies on NA, grey colonies on EMBA	+R	+	-	+	<i>Bacillus cereus</i>
Smooth moist and shiny yellow colonies on NA	+S	-	-	-	<i>Micrococcus luteus</i>
Small circular moist and shiny cream colonies on NA	+S	-	-	-	<i>Enterococcus faecalis</i>
Moist and shiny light pink colonies on EMBA	-R	-	-	-	<i>Enterobacter cloaca</i>
Small smooth moist and shiny	-R	+	-	-	<i>Escherichia coli</i>

greenish metallic sheen colonies on EMBA					
Smooth shiny fish eye colonies on SSA	-R	+	-	-	<i>Salmonella typhimurium</i>
Shiny pink colonies on SSA	-R	-	-	-	<i>Shigella dysenteriae</i>
small smooth moist and shiny red colonies on NA	-R	-	-	-	<i>Serratia marcescens</i>
Rough mucoid and slimy cream colonies on NA	+R	+	-	+	<i>Bacillus subtilis</i>
Dull circular umbonate cream colonies on NA	+S/R	-	-	-	<i>Corynebacterium</i> sp
Smooth mucoid and slimy pink colonies on EMBA	-R	+	+	-	<i>Klebsiella pneumonia</i>
Smooth moist and shiny golden yellow colonies in NA and yellow colonies on MSA	+S	-	-	-	<i>Staphylococcus aureus</i>

The total count of fungi isolated from samples of locally brewed burukutu from different locations and their colonial characterization on Potato Dextrose Agar is shown in Table 3. Colony counts is between 7.0×10^8 - 1.14×10^{10} . *Saccharomyces* species is dominant compared to *Rhizopus stolonifer* and *Mucor* species. Percentage occurrence of microorganisms (bacteria and fungi) isolates from the samples is shown in Table 4.

Table 3. Total count and morphological characteristics of fungal isolates on Potato dextrose agar (PDA)

Sample Location code	Colony Count (cfu/ml)	Colony type	Morphological characteristics	Most probable identity
OBZA	2.0×10^9	OBZA _x	Tall filamentous hyphae bearing black spores at its tip.	<i>Rhizopus stolonifer</i>
		OBZA _y	Smooth moist and shiny creamy white colonies.	<i>Saccharomyces cerevisiae</i>
IHAG	3.2×10^9	IHAG _x	Smooth, moist and shiny creamy white colonies.	<i>Saccharomyces cerevisiae</i>
		IHAG _y	Tall filamentous hyphae bearing black spores at its tip.	<i>Rhizopus stolonifer</i>

NEKD	7.0×10^8	NEKD _x	Short white filamentous cotton wool like hyphae.	<i>Mucor</i> spp
		NEKD _y	Spherical, raised, cream colored colonies.	<i>Saccharomyces cerevisiae</i>
EZBO	1.14×10^{10}	EZBO _x	Tall, filamentous hyphae with black spores at tips.	<i>Rhizopus stolonifer</i>
		EZBO _y	Spherical, raised, cream colored colonies.	<i>Saccharomyces cerevisiae</i>
		EZBO _z	Short white filamentous cotton wool like hyphae.	<i>Mucor</i> spp
OWER	1.4×10^9	OWER _x	Spherical, raised, cream colored colonies.	<i>Saccharomyces cerevisiae</i>
		OWER _y	Mucoid cream colonies. Gram positive oval budding cells.	<i>Saccharomyces cerevisiae</i>

Table 4. Percentage occurrence of microorganisms isolated from samples

Bacterial Isolates	Number of colonies	Percentage occurrence (%)	Fungal Isolates	Number of Colonies	Percentage occurrence (%)
<i>Escherichia coli</i>	51	9.7	<i>Saccharomyces</i> Spp	118	60.2
<i>Enterobacter cloaca</i>	23	4.4	<i>Mucor</i> spp	35	17.9
<i>Enterococcus faecalis</i>	134	25.5	<i>Rhizopus stolonifer</i>	43	21.9
<i>Micrococcus luteus</i>	48	9.1			

<i>Bacillus cereus</i>	67	12.7			
<i>Salmonella typhimurium</i>	15	2.9			
<i>Corynebacterium sp</i>	11	2.1			
<i>Shigella dysenteriae</i>	34	6.5			
<i>Staphylococcus aureus</i>	69	13.1			
<i>Serratia marcescens</i>	12	2.3			
<i>Klebsiella pneumoniae</i>	22	4.1			
<i>Bacillus subtilis</i>	43	8.2			

Escherichia coli is most prevalent at 34.1% followed by *Enterobacter* spp (20.3%) while *Micrococcus* species the least occurrence at 10.8%. For the fungal isolates, *Saccharomyces* spp had the highest occurrence at 50.0% while *Rhizopus* species had the least at 18.2%.

Table 5 shows pH and titratable acidity of the locally fermented alcoholic beverages, *burukutu*. pH is acidic, between 3.90 to 4.71, while the TTA ranges between 2.99 and 3.33. Temperature is maintained at ambient (27.0°C to 30.0°C). Antioxidant and phytochemical compositions of the beverages is shown in Table 6 and Table 7 respectively. Free scavenging radicals in the beverage is 0.064 - 0.071. Tannins and alkaloids are significant in the beverages. Other phytochemicals present in small quantities includes flavonoids, anthocyanins, saponins and glycosides.

Table 5. Hydrogen ion (pH) concentration and Titratable acidity (TTA) of locally fermented alcoholic beverage, *Burukutu* (n=3)

Samples (<i>Burukutu</i>)	pH	Solution temperature (°C)	TTA (g/L)
Bk1	4.21±0.01	27.1±0.01	3.11±0.02
Bk2	3.90±0.01	28.0±0.01	3.21±0.02
Bk3	4.22±0.01	28.1±0.01	2.99±0.03
Bk4	4.00±0.01	30.0±0.03	3.00±0.01
Bk5	4.44±0.03	26.6±0.02	3.11±0.01

Bk6	4.63±0.01	26.1±0.05	3.33±0.05
Bk7	4.43±0.01	27.1±0.01	3.00±0.01
Bk8	4.71±0.05	26.0±0.02	3.10±0.05
Bk9	4.00±0.01	28.2±0.01	3.20±0.01
Bk10	4.21±0.01	27.0±0.01	3.00±0.05

Table 6. Antioxidants composition of alcoholic beverage, *Burukutu* (n=3)

Samples (<i>Burukutu</i>)	Absorbance	% RSA
Bk1	1.007 SD±0.03	0.066 SD±0.03
Bk2	1.000 SD±0.01	0.067 SD±0.01
Bk3	0.879 SD±0.01	0.071 SD±0.01
Bk4	0.981 SD±0.02	0.067 SD±0.02
Bk5	1.074 SD±0.02	0.064 SD±0.02
Bk6	1.002 SD±0.02	0.064 SD±0.02
Bk7	1.078 SD±0.01	0.067 SD±0.01
Bk8	0.911 SD±0.01	0.070 SD±0.01
Bk9	1.002 SD±0.05	0.067 SD±0.05
Bk10	1.005 SD±0.01	0.067 SD±0.01

Absorbance of control of DPPH, 3000.

Table 7. Quantitative Phytochemical composition of alcoholic beverage, *Burukutu* (n=3)

Samples (<i>Burukutu</i>)	Tan	Sap	Alk	Flav	Ant	Gly
Bk1	1.021±0.02	0.021±0.02	0.991±0.01	0.012±0.01	0.008±0.05	0.010±0.05
Bk2	1.000±0.01	0.031±0.01	1.003±0.01	0.023±0.03	0.013±0.01	0.014±0.01
Bk3	0.992±0.01	0.041±0.03	0.931±0.02	0.021±0.04	0.021±0.02	0.021±0.01
Bk4	0.110±0.05	0.024±0.01	1.112±0.02	0.021±0.04	0.016±0.03	0.031±0.01
Bk5	0.661±0.01	0.011±0.03	0.081±0.02	0.033±0.04	0.008±0.01	0.044±0.05
Bk6	0.512±0.02	0.033±0.03	0.090±0.04	0.033±0.05	0.012±0.02	0.019±0.01
Bk7	1.009±0.04	0.030±0.01	0.071±0.01	0.021±0.01	0.033±0.01	0.008±0.02
Bk8	0.542±0.01	0.022±0.01	1.002±0.05	0.023±0.01	0.012±0.01	0.012±0.01
Bk9	0.431±0.01	0.022±0.01	1.111±0.01	0.051±0.01	0.017±0.01	0.011±0.01
Bk10	1.110±0.05	0.024±0.01	0.975±0.02	0.018±0.01	0.017±0.01	0.019±0.03

Tan, tannin; Sap, saponin; Alk, alkaloid; Flav, flavonoid; Ant, anthocyanin; Gly, glycosides.

4.0. Discussions

Microorganisms associated with traditionally fermented alcoholic beverages had been reported independently by Elmahmood & Doughari (2007), Kowawole *et al.* (2018), Eze *et al.* (2012) and Ire *et al.* (2020) amongst others. Mbajiuka *et al.* (2010) also reported the presence of *Escherichia coli* and species of *Enterobacter*, *Bacillus*, *Micrococcus*, *Enterococcus*, *Staphylococcus*, *Streptococcus*, *Proteus* and *Pseudomonas*.

Escherichia coli is a significant member of the coliform group of bacteria and is typically found in the intestinal tract of both humans and vertebrates as a part of their normal flora. However, it's important to note that certain strains of *Escherichia coli* and *Enterococcus faecalis* have the potential to cause health issues, including gastroenteritis, urinary tract infections, and diarrhea in infants and young children, as observed in the study by Kolawole *et al.* (2018).

The presence of *Escherichia coli* in various samples can serve as an indicator of faecal contamination. This contamination may arise due to various factors, such as inadequate sanitation during processing, unhygienic water sources, particularly in areas where clean drinking water is scarce, the use of non-sterilized utensils, and contamination by flies.

Bacillus species are Gram-positive aerobic spore formers. Most members of the genus are saprophytic organisms prevalent in soil, water, air and on vegetation. *Bacillus cereus* and *Bacillus subtilis* are the most encountered species in the group. *Bacillus cereus* when grown on food causes food poisoning by production of an enterotoxin (Eze *et al.*, 2012).

Staphylococcus aureus produce enterotoxins associated with food borne intoxication and staphylococcal scalded skin syndrome (SSSS) in humans (Jay *et al.*, 2005). *Proteus mirabilis* and *Pseudomonas aeruginosa* are Gram negative bacteria that produces enzymes involved in proteolysis and food spoilage even at refrigeration temperature (Eze *et al.*, 2012; Jay *et al.*, 2005).

The fungal isolates identified across the samples include *Saccharomyces* species, *Mucor* species and *Rhizopus* species. This is similar to the reports of Kolawole *et al.* (2018), Mbajiuka *et al.* (2010), Bala *et al.* (2017) and Abbas *et al.* (2020).

The presence of fungi may be attributed to the acidic nature of the sample since it has been observed that yeasts and moulds are capable of utilizing organic acids. Also the presence of fungi in the beverage may lead to poisoning caused by fungi result in the production of mycotoxins (Jay *et al.*, 2005) and undesirable odour, colour changes and even taste of the samples (Malomo & Poppola, 2020).

The presence of fungi could result from the nutritional composition of the millet and sorghum which are major raw materials. *Saccharomyces* species are associated with the fermentation of cereals (Mbajiuka *et al.*, 2010; 2020; Abbas *et al.*, 2020).

The alcoholic beverage is rich in some phytochemicals and antioxidants. Phytochemicals are biologically active organic substances found in plants used by humans as food, which may be beneficial for health, but for which no specific human deficiency disorder has been identified. The importance of bioactive compounds like alkaloids,

phenolics, flavonoids and some other secondary metabolites have been reported by Edori *et al.* (2019). The presence of these phytochemicals is an added advantage to the dental formulations. These phytochemicals have been reported to exert strong antibacterial activity against several microbes associated with oral diseases (Adeyemi *et al.*, 2004). Antioxidants have the ability to scavenge free radicals in the human body and have been suggested to contribute to the protective effect of plant-based foods on diseases (Stanner & Weichselbaum 2013).

5.0. Conclusion and Recommendation

The high microbial counts and groups of microorganisms recorded in *Burukutu* samples could be attributed to the chains of production processes from the raw materials (sorghum and millet) to the packaging and storage. Food spoilage and pathogenic microorganisms observed are associated with low hygiene levels. This situation raises serious concerns for the health of consumers.

Launching of comprehensive awareness campaigns targeting communities, producers, vendors, and consumers of *Burukutu* is strongly recommended. The focus of these campaigns is to educate producers and consumers about the health risks associated with consuming the beverage when adherence to proper manufacturing and hygiene practices is lacking.

Burukutu producers and vendors can be encouraged to seek technical guidance and support from regulatory bodies such as the National Agency for Food, Drug Administration and Control (NAFDAC). This collaboration can facilitate the implementation of stringent quality standards and ensure that *Burukutu* production adheres to recommended hygiene protocols, thereby safeguarding the health of consumers.

Declarations

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Competing Interests Statement

The authors declare no competing financial, professional, or personal interests.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

All the authors took part in literature review, analysis and manuscript writing equally.

Availability of data and material

All data pertaining to the research is kept in good custody by the authors.

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